Antimicrobial Susceptibility Patterns in Montana: A Survey of Laboratories in 2002

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Antimicrobial Susceptibility Patterns Reported by Laboratories in Montana, 2002

Background

Antimicrobial agents or antibiotics, after their discovery in the 1940's, transformed the medical community's ability to reduce illness and death from infectious diseases. However, over the decades, pathogens have developed resistance to antimicrobial agents. Although antimicrobial resistance (AMR) can be considered a natural response to the selective pressure of using antimicrobial agents, it is exacerbated by several factors, including abuse, under-use or misuse of antimicrobials, poor patient compliance, and poor quality of available drugs. Unfortunately, virtually all important bacterial infections in the United States and throughout the world are becoming resistant to the primary antimicrobials used to treat them, including infections of public health importance such as pneumonia, diarrheal diseases, and tuberculosis. The emergence and spread of antimicrobial resistance is now threatening to undermine our ability to treat infections and save lives to such an extent that antimicrobial resistance has been deemed one of the world's most pressing public health problems.

To address the pressing public health issue of AMR, multiple federal agencies developed "A Public Health Action Plan to Combat Antimicrobial Resistance". One of the top priorities identified in the Action Plan is to implement surveillance for AMR. Surveillance of AMR is critical in providing an early warning of emerging problems, monitoring changing patterns of resistance, and targeting and evaluating prevention and control measures.

In 2003, the State of Montana Department of Public Health and Human Services Public Health Laboratory (PHL) surveyed laboratories in the State of Montana regarding antimicrobial resistance. This survey was undertaken as a follow-up to a similar survey conducted in 1996 that collected data about reported AMR patterns during that year. Data from the 1996 survey indicated that levels of antimicrobial susceptibility in Montana were comparable to national susceptibility data; however, there were some unusual results reported, including several vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates. Further investigation into the reported VRSAs showed with the possibility of three exceptions, the cases were not resistant upon re-testing. It was suggested that automated MIC equipment had under-dosed the specimens and yielded false positive results. The purpose of the 2003 survey was to: (1) assess the AMR patterns reported for select bacterial isolates in 2002, (2) to determine if overall antimicrobial susceptibility patterns had changed since the previous survey, and (3) to determine if unusual reports continued to occur.

Methods

Survey

In March 2003, the PHL sent a questionnaire (Appendix 1) to all licensed hospital and clinic laboratories in the State of Montana that were known or presumed to conduct microbiologic testing. Labs were asked to report the number of isolates of five microorganisms that they tested for AMR from January 1, 2002 through December 31, 2002; they were also asked to include a copy of their antibiogram from the same time frame.

The survey also included questions about which antimicrobial susceptibility testing (AST) methods were utilized, what basis was used for the interpretation for AST results, which standard references were used to guide AST results, and how personnel were trained to do

AST methods. The responses from these questions will be used to provide targeted training to laboratory professionals on AST.

To maximize the response rate from the survey, a two-tier approach was used. First, the questionnaire was sent to 76 laboratories throughout the state. A follow-up telephone call was placed to further prompt those laboratories that did not respond to the questionnaire or returned the questionnaire without an antibiogram attached.

Analysis of data

Data from the questionnaire and antibiograms were combined in a database and descriptive statistics were generated using statistical software (SPSS). The total number of isolates reported for each organism was determined and AST methods were summarized by organism across laboratories that performed testing. For each microorganism, the number of laboratories that performed AST testing and the number of labs that reported finding resistance was calculated by antimicrobial agent.

The percent of organisms resistant to an antimicrobial was calculated using two different criteria and presented in two formats: percent resistant (aggregate) and the median percent resistant (reported). For labs that reported the number of resistant organisms found and the total numbers of isolates tested, the percent resistant was calculated by aggregating these data for all laboratories and reported as percent resistant (aggregate). However, some laboratories only reported the percent of organisms tested that showed AMR. Therefore, the median percent resistant (reported) was calculated as the median value of percent resistant values reported by all laboratories that performed AST testing. The range of the number of isolates tested and range of the percent resistance (reported) were also calculated for those labs that reported finding resistance to an agent.

To compare the prevalence of AMR over time in the state, data from the 1996 survey was obtained and a list of laboratories participating in both the 1996 and the 2003 survey was compiled. Subsets of both data sets were derived for the organisms and antimicrobial agents that the two surveys had in common; these data were then analyzed to generate comparative statistics for AMR over time.

Results and Discussion

Survey

Of the 76 laboratories contacted, 42 returned the questionnaire and 13 confirmed, by telephone, that AST testing was not done by their facility. This process resulted in 55 of the 76 laboratories completing the survey for an overall response rate of 72.4%. Of the 55 responding laboratories, 18 (32.7%) did not perform microbiology or AST testing. Of the 37 laboratories the performed microbiology, 22 (59%) submitted an antibiogram.

Testing methodology

For laboratories that performed microbiology and/or AST, the number of isolates and the primary method used for determining AST are presented for select Gram positive bacteria (Table 1) and Gram negative bacteria (Table 2).

Table 1. Number of gram positive bacterial isolates tested and the methods of testing for antimicrobial susceptibility reported by laboratories in Montana, 2002.

	Gram Positive Organisms		
	*S. pneumoniae**	*Enterococcus* sp.	*S. aureus*
Number of labs reporting >1 isolate tested	19	36	36
Isolates			
Total number	154	3,872	6,047
Range	1 – 31	1 – 494	12 – 792
25^{th} Percentile	2.0	18.5	42.3
75^{th} Percentile	14.0	171.3	268.5
Primary Testing Method†	n (%)	n (%)	n (%)
MIC, automated	4 (11)	28 (76)	28 (77)
MIC, manual	4 (11)	5 (14)	4 (11)
Disk diffusion	11 (31) **	3 (8)	4 (11)
Etest	2 (6)	--- ---	--- ---
Combination	7 (20)	--- ---	--- ---
Referred	5 (14)	--- ---	--- ---
Secondary Testing Method†			
Disk diffusion	4 (29)	--- ---	--- ---
Other	7 (50)	--- ---	--- ---
Combination	3 (21)	--- ---	--- ---
Vancomycin screen agar	--- ---	5 (28)	--- ---
Vancomycin disk diffusion	--- ---	3 (17)	--- ---
Vancomycin Etest	--- ---	1 (5.6)	--- ---
Other	--- ---	6 (33)	--- ---
Combination	--- ---	3 (17)	--- ---
Oxacillin salt agar screen	--- ---	--- ---	5 (26)
Vancomycin screening agar	--- ---	--- ---	0 (0)
Disk diffiusion	--- ---	--- ---	4 (21)
Etest	--- ---	--- ---	0 (0)
Other	--- ---	--- ---	5 (26)
Combination	--- ---	--- ---	5 (26)
Laboratories that perform confirmatory AST	14 (74)	18 (50)	19 (53)

* Blood and CSF isolates only

† Column percents may total > 100% if laboratories selecting more than one category or due to rounding error; or < 100% if some laboratories did not respond to this item

** Oxacillin for penicillin susceptibility

Table 2. Number of gram negative bacterial isolates tested and the methods of testing for antimicrobial susceptibility reported by laboratories in Montana in 2002.

	Gram Negative Organisms	
	E.coli/K.pneumoniae	*P. aeruginosa**
Number of labs reporting $\geq$1 isolate tested	37	14
Isolates		
Total number	20,011	59
Range	13 – 2665	1 – 17
25th Percentile	95.5	1.0
75th Percentile	860.5	4.3
Primary Testing Method†	n (%)	n (%)
MIC, automated	28 (76)	27 (75)
MIC, manual	5 (14)	5 (14)
Disk diffusion	4 (11)	3 (8)
Referred	--- ---	1 (3)
Secondary Testing Method†		
Disk diffusion for ESBLs w/wo clavulinic acid	3 (27)	--- ---
Etest for CT/CTL, TZ/TZL	0 (0)	--- ---
Other	8 (73)	6 (100)
Laboratories that perform confirmatory AST	11 (30)	6 (43)

*Blood and CSF isolates only

† Column percents may total > 100% if laboratories selecting more than one category or due to rounding error; or < 100% if some laboratories did not respond to this item.

The above information shows that most laboratories that report performing AST are using automated MIC testing, with the exception of testing *S. pneumoniae*. However, few laboratories are performing confirmatory testing on organisms with potentially significant resistance when it does occur.

All 37 of the laboratories that performed microbiology provided information about their possession of current NCCLS manuals and how AMR reporting rules, if in place, were developed. A laboratory was considered to have a NCCLS manual if they reported that the manual was either in their current possession or on order.

Table 3. Number of laboratories access to NCCLS manuals and with antimicrobial resistance reporting rules, Montana, 2002.

Laboratories Reporting Having NCCLS Documents	Yes†	
Manual	n	(%)
M2A8 Disk diffusion	13	(35)
M7 – A6 – MIC	12	(32)
M100 – S13	14	(38)
M39 – A	10	(27)
Antimicrobial Resistance Reporting Rules		
Laboratories with Antimicrobial Resistance Reporting Rules in Place	6	(16)
Resources Used to Formulate Reporting Rule		
NCCLS rules	10	(29)
Formulary	1	(3)
Combination	21	(60)
Other	3	(7)

† Column percents may not total 100% due to rounding error.

The data above shows that although virtually all laboratories that responded may use NCCLS documents to establish their reporting rules, only about one-third of the laboratories actually have access to the manuals. However, the majority of laboratories utilize automated testing technologies that most likely have the NCCLS standards built-in; therefore, labs have indirect access to these important criteria.

Thirty-six laboratories provided information as to the number of staff trained to perform AST. The number of staff ranged from 2 – 12 persons trained to perform AST; of these staff, 0 – 100% were reported to be cross-trained to perform laboratory testing in areas other than microbiology.

Antimicrobial resistance patterns and comparison of AMR trends over time (by organism)

A total of 13 laboratories, as identified by matching the facility names reported in both questionnaires, responded to both the 1996 survey and the 2003 survey. Organisms that were included on both surveys were *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Enterococcus spp*. Antimicrobial agents that were common to the two surveys were included in the analysis of comparative data for comparison of resistance patterns over time.

Steptocococcus pneumoniae Penicillin has long been the therapeutic agent of choice for infections with *Streptococcus pneumoniae*. Over the past decade, the incidence of infections with penicillin resistant strains has risen; this has been coupled with resistance to third-generation cephalosporins. This change in susceptibility of *S. pneumoniae* to beta-lactam antimicrobial agents has created a major challenge in the therapy of invasive infections by this common pathogen.

Resistance of *S. pneumoniae* to penicillin is considered to be intermediate if the minimal inhibitory concentration (MIC) is 0.1 to1.0ug/mL and high level if the MIC is greater than l.0ug/mL. Pneumococci are intermediately resistant to cefotaxime and ceftriaxone if the MIC is 0.5 to 1.0ug/mL and highly resistant if the MIC is greater than 1.0 ug/mL. The distinction is of practical importance because infection with intermediately resistant strains usually can be cured with beta-lactam antibiotics if the infection is at a body site where the antibiotic is able to penetrate to reach concentrations substantially in excess of the MIC. However, meningitis with *S. pneumoniae* intermediately resistant to penicillin and cephalosporins should not be treated with these agents because bactericidal concentrations of the drug in the cerebrospinal fluid may not be attained. Fortunately, all pneumococcal strains that are resistant of penicillin and/or cephalosporins currently are susceptible to vancomycin.

The levels of resistance to select antimicrobials reported for *Streptococcus pneumoniae* during 2002 are presented in Table 4. A comparison of the levels of resistance to select antimicrobials for *Streptococcus pneumoniae* are presented in Table 5.

For 2002, significant levels of resistance to penicillin (18-24%) reinforces the standard that all isolates should be tested. Fortunately, resistance to vancomycin was not noted during this period. However, culture submission or testing practices may play a role in the wide variability (15 – 49%) among the percent resistance (reported); i.e. laboratories may selectively test penicillin resistance for oxacillin-resistant isolates or isolates from sterile body sites.

For some of the antimicrobial agents that were included on both the 1996 and 2003 surveys, the levels of resistance has remained stable over the 6-year time period; however, the levels of resistance to ciprofloxacin, erythromycin, and penicillin has increased slightly over this period. These results may reflect differences in testing methodologies, culture submission practices, changes in antimicrobial usage patterns, or be true increases in resistance levels over time. In the case of ciprofloxacin, very few labs tested a small number of isolates; therefore, this estimate of resistance is subject to those limitations.

Table 4. Antimicrobial resistance patterns reported by Montana laboratories for *Streptococcus pneumoniae* isolates, 2002.

Antimicrobial Agent	Number of laboratories		% Resistant (aggregate)†		Median (%) resistant (reported)‡	Range (for labs reporting resistance)	
	Testing for antimicrobial resistance	Reporting resistance to agent	% Resistant	# Resistant/total # isolates tested		# Isolates tested§	% Resistant (reported)
Cefotaxime	5	2	23	(3/13)	0	NR - 7	6 - 43
Ceftriaxone	8	3	5	(12/249)	0	7 - 58	8 - 50
Cefuroxime	1	1	11	(11/100)	11	--- ---	--- ---
Ciprofloxacin	1	1	50	(4/7)	50	--- ---	--- ---
Erythromycin	9	8	27	(86/319)	18	NR - 100	15 - 69
Levofloxacin	7	4	5	(15/306)	2	45 - 100	2 - 15
Penicillin	7	5	24	(65/267)	18	NR - 100	15 - 49
Tetracycline	7	4	2	(24/286)	2	45 - 100	2 - 15
Vancomycin	8	0	0	(0/286)	0	--- ---	--- ---

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
§NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.

Table 5. Comparison of antimicrobial resistance patterns reported by Montana laboratories for *Streptococcus pneumoniae* isolates, 1996 and 2002.

Antimicrobial Agent	1996					2002				
	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡
	Testing for AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates		Testing for AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates	
Cefotaxime	---	---	---	---	---	3	2	43	(3/7)	6
Ceftriaxone	1	1	3	(1/32)	3	6	3	5	(12/243)	4
Cefuroxime	---	---	---	---	---	1	1	11	(11/100)	11
Ciprofloxacin	2	2	8	(8/99)	14	1	1	50	(4/7)	50
Erythromycin	3	3	8	(12/150)	7	6	6	30	(72/243)	23
Levofloxacin	---	---	---	---	---	6	3	6	(14/236)	1
Penicillin	4	4	28	(57/206)	11	5	5	41	(96/236)	34
Tetracycline	1	1	12	(6/51)	12	4	3	9	(19/210)	6
Vancomycin	1	0	0	(0/51)	0	5	0	0	(0/286)	0

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.

Staphylococcus aureus *Staphylococcus aureus* strains resistant to methicillin and cloxacillin pose a serious clinical and public health problem, as they can be transmitted from patient to patient in hospitals as well as in community settings; many strains appear to be acquired nosocomially. Resistance (like that of the pneumococcus to penicillin) is mediated by a modification in the penicillin-binding protein to which methicillin normally binds. Methicillin-resistant *S. aureus* strains are also resistant to cephalosporins regardless of in vitro susceptibility testing results. Strains resistant to methicillin are also often resistant to erythromycin, clindamycin, aminoglycosides, tetracycline and chloramphenicol. Intravenous vancomycin is the drug of choice for such infections. Infections should be recognized and treated, and infection control precautions instituted as early as possible, to prevent nosocomial spread.

The levels of resistance to select antimicrobials for *Staphylococcus aureus* in 2002 are presented in Table 6. A comparison of the levels of resistance to select antimicrobials for *Staphylococcus aureus* are presented in Table 7.

For 2002, high levels of penicillin-resistant S. aureus were reported (90-91%); however, this may reflect a sampling bias for this organism due to inclusion of screening isolates. The wide variability (33-100%) in the percent resistant (reported) would suggest that sampling bias may play a role in this finding. It is also noteworthy that, although 0% of isolates were resistant to vancomycin (aggregate and reported), 6 of the 4518 tested and 1-2% of those reported resistant were found “resistant” to vancomycin.   Unfortunately, it is impossible to discern the reporting facilities subsequent actions upon this finding from this survey.

For most of the antimicrobial agents that were included on both the 1996 and 2003 surveys, the levels of resistance has remained stable over the 6-year time period; however, the level of resistance to ciprofloxacin has increased slightly over this period. These results may reflect differences in testing methodologies, culture submission practices, changes in antimicrobial usage patterns, or be true increases in resistance levels over time. Testing for resistance to both antimicrobials was performed in several labs and a substantial number of isolates were tested; therefore, this trend merits further monitoring. For those laboratories that responded to both surveys, there was a reduction in the numbers of “vancomycin-resistant” organisms reported, indicating that previous educational efforts may have been successful.

Table 6. Antimicrobial resistance patterns reported by Montana laboratories for *Staphylococcus aureus* isolates, 2002.

Antimicrobial Agent	Number of laboratories: Testing for antimicrobial resistance	Number of laboratories: Reporting resistance to agent	% Resistant (aggregate)[†]: % Resistant	% Resistant (aggregate)[†]: # Resistant/total # isolates tested	Median (%) resistant (reported)[‡]	Range (for labs reporting resistance): # Isolates tested[§]	Range (for labs reporting resistance): % Resistant (reported)
Cefazolin[∝]	20	18	24	(1063/4462)	24	NR - 791	1 - 100
Ciprofloxacin	15	15	21	(549/2647)	20	3 - 498	3 - 100
Clindamycin	20	19	12	(515/4167)	15	NR - 791	2 - 59
Gentamicin	20	14	2	(65/4238)	1	NR - 791	1 - 5
Oxacillin	21	18	23	(1032/4518)	21	15 - 791	7 - 100
Penicillin	17	17	90	(3583/3989)	91	NR - 791	33 - 100
Rifampin	17	9	1	(47/3581)	0	NR - 791	1 - 5
Tetracycline	20	18	3	(158/4518)	4	NR - 791	2 - 33
Trimethoprim/ Sulfamethoxazole	18	8	1	(36/4196)	0	NR - 791	1 - 19
Vancomycin	20	3	0	(6/4518)	0	46 - 392	1 - 2

[†]Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
[‡]Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
[§]NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.
[∝]The tests for this antimicrobial agent may not appropriate to report (per NCCLS) or use for clinical decision-making. However, the result was reported on the antibiogram by the reporting laboratory, it may or may not have been reported to the isolate submitter.

Table 7. Comparison of antimicrobial resistance patterns reported by Montana laboratories for *Staphylococcus aureus* isolates, 1996 and 2002.

Antimicrobial Agent	1996					2002				
	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡
	Testing for AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates		Testing For AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates	
Ciprofloxacin	13	12	9	(211/2281)	9	9	9	26	(339/1675)	26
Clindamycin	12	12	10	(215/2185)	10	12	11	12	(293/2391)	15
Gentamicin	11	9	3	(50/1860)	3	11	8	1	(33/2462)	1
Oxacillin	13	13	17	(378/2281)	11	11	11	26	(719/2742)	21
Tetracycline	13	13	8	(175/2281)	7	12	11	4	(101/2742)	4
Vancomycin	13	4	0	(11/2281)	1	12	1	0	(1/2742)	0

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.

Enterococci spp. Only 20 years ago, controversy existed about whether enterococci were common causes of nosocomial infections or commensals in the ICU. Then in 1997 data showed that enterococci comprised 16% of blood-borne isolates in adult patients cared for in medical ICUs. The two species of primary concern to public health today are *E. faecium* and *E. faecalis*. Enterococci develop resistance through acquired mechanisms, which include gene transcription and acquisition of DNA via plasmids and transposons (genetic elements that can move from one location on DNA to another within and between bacteria), and through intrinsic mechanisms. Intrinsic resistance is a natural property of enterococci and does not require a change in the bacteria or previous exposure to antibiotics. Enterococci are intrinsically resistant to cephalosporins as a drug class and some species of enterococci, such as *E. gallinarum* and *E. casseliflavus/E. flavescens* are intrinsically resistant to vancomycin. Vancomycin resistant enterococci (VRE) appeared in the mid-1980s after a period of increasing prophylactic and therapeutic use of vancomycin. It is suspected that widespread use of antibiotics with poor activity against VRE promoted emergence of these pathogens. Effective therapeutic options for VRE infections are limited. In some instances of deep-seated VRE infection, high-dose ampicillin or ampicillin-sulbactam may retain clinical efficacy if the MIC for ampicillin is <64ug/mL. Gentamicin or streptomycin should be added unless the enterococci are highly resistant to these antibiotics. There are new antibiotics out but they are not bactericidal and their roles have not been clearly defined.

The levels of resistance to select antimicrobials for *Enterococcus* species are presented in Table 8. A comparison of the levels of resistance to select antimicrobials for *Enterococcus spp.* isolates from 1996 and 2002 are presented in Table 9.

Of the 13 laboratories that test enterococci for resistance to vancomycin, 4, (31%) reported finding resistance. However, the total reported resistance remains low (2%). Because laboratories were asked to report resistance patterns for all *Enterococci spp*., it is unknown if species that are intrinsically resistant to vancomycin or the other antimicrobial agents influence these findings. For the antimicrobial agents that were included on both the 1996 and 2003 surveys, the levels of resistance have remained stable over the 6-year time period.

Table 8. Antimicrobial resistance patterns reported by Montana laboratories for *Enterococcus species* isolates, 2002.

Antimicrobial Agent	Number of laboratories		% Resistant (aggregate)†		Median (%) resistant (reported) ‡	Range (for labs reporting resistance)	
	Testing for antimicrobial resistance	Reporting resistance to agent	% Resistant	# Resistant/total # isolates tested		# Isolates tested§	% Resistant (reported)
Ampicillin	16	11	5	(107/2350)	1	3 - 416	1 - 90
Ciprofloxacin	15	14	40	(758/1898)	42	1 - 416	20 - 100
Levofloxacin	17	17	32	(934/2882)	33	NR - 416	12 - 100
Penicillin	17	14	6	(172/2791)	2	NR - 416	1 - 100
Tetracycline	19	19	70	(2088/2983)	66	1 - 416	25 - 100
Vancomycin	21	10	2	(62/3046)	0	10 - 416	1 - 100

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
§NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.

Table 9. Comparison of antimicrobial resistance patterns reported by Montana laboratories for *Entercoccus species* isolates, 1996 and 2002.

Antimicrobial Agent	**1996**					**2002**				
	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡	Number of laboratories		Resistance (aggregate) †		Median % resistant (reported) ‡
	Testing for AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates		Testing for AMR	Reporting resistance to agent	Percent resistant	# Resistant/ total # isolates	
Ciprofloxacin	10	10	43	(575/1343)	27	10	10	39	(546/1416)	37
Penicillin	7	7	8	(62/793)	5	12	10	6	(124/2006)	3
Tetracycline	10	10	78	(930/1196)	80	12	12	71	(1448/2040)	70
Vancomycin	12	7	1	(19/1713)	1	13	6	1	(26/2067)	0

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.

Escherichia coli* and *Klebsiella pneumoniae *E. coli* and *Klebsiella pneumoniae* are the most common gram-negative pathogens that infect hospitalized patients. Their management has become complicated by their generation of a variety of beta-lactamases. Because of variability of laboratory testing policies for extended spectrum beta-lactamases (ESBLs), drug susceptibility patterns of ESBL-producing organisms may be reported inaccurately. Most laboratories report strains of *K. pneumoniae* and *E. coli* with a MIC <8ug/mL as susceptible to ceftazidime. Bacterial strains with ceftazidime MIC values of 2-4 ug/mL, however, may produce ESBLs. The existance of ESBLs should be suspected when enterobacteriaceae have ceftazidime MICS> 2 ug/mL. Unfortunately, most laboratories do not report specific MIC values and simply indicate that bacteria with MIC values < 8ug/mL are "susceptible." Because ceftazidime is the most susceptible of the extended-spectrum cephalosporins to undergo hydrolysis by ESBLs, ESBLs should be suspected when *K. pneumoniae* and *E. coli* exhibit ceftazidime resistance. E. coli is responsible for three types of infections in humans: urinary tract infections, neonatal meningitis, and intestinal diseases. The vast majority of *Klebsiella* infections are associated with hospitalization. They are opportunistic organisms and primarily attack immunocompromised individuals.

The levels of resistance to select antimicrobials for *E. coli* and *Klebsiella pneumoniae* are presented in Table 10 and 11, respectively.

Less than one-third of laboratories that tested for ESBLs reported finding them and very low levels of ESBLs were reported: 1-10% for E. coli (0% aggregate) and 1 – 3% for K. pneumoniae (1% aggregate).

Table 10. Antimicrobial resistance patterns reported by Montana laboratories for *Escherichia coli* isolates, 2002.

Antimicrobial Agent	Number of laboratories		% Resistant (aggregate)†		Median (%) resistant (reported) ‡	Range (for labs reporting resistance)	
	Testing for antimicrobial resistance	Reporting resistance to agent	% Resistant	# Resistant/total # isolates tested		# Isolates tested§	% Resistant (reported)
Ampicillin	21	21	34	(3832/11367)	33	NR - 2166	20 - 100
Ampicillin/ Sulbactam	16	15	30	(2484/8412)	29	NR - 2166	10 - 41
Amikacin	15	2	0	(3/7228)	0	10 - 193	1 - 10
Cefazolin	22	21	7	(827/11735)	5	NR - 2166	3 - 37
Cefotetan	11	2	0	(18/5620)	0	855 - 989	1 - 1
Cefoxitin	4	3	2	(78/3467)	4	135 - 973	2 - 12
Ceftazidime	18	5	0	(24/9268)	0	NR - 989	1 - 10
Ceftriaxone	20	2	0	(10/9558)	0	NR - 973	1 - 100
Cefuroxime	12	10	4	(241/6762)	2	NR - 2166	1 - 10
Ciprofloxacin	20	17	2	(253/10470)	1	46 - 2166	1 - 7
Gentamicin	22	21	2	(247/11736)	2	NR - 2166	1 - 100
Imipenem	15	0	0	(0/9082)	0	--- ---	--- ---
Levofloxacin	16	13	2	(252/10407)	2	NR - 2166	1 - 4
Piperacillin	15	15	25	(2091/8251)	25	NR - 2166	10 - 37
Piperacillin/ Tazobactam	11	7	2	(157/7040)	2	NR - 2166	2 - 4
Tetracycline	11	11	16	(1078/6805)	16	NR - 2166	6 - 30
Tobramycin	19	14	1	(151/11014)	1	NR - 2166	1 - 100
Trimethoprim/ Sulfamethoxazole	21	20	14	(1607/11601)	14	NR - 2166	7 - 67

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
§NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.

Table 11. Antimicrobial resistance patterns reported by Montana laboratories for *Klebsiella pneumoniae* isolates, 2002.

Antimicrobial Agent	Number of laboratories		% Resistant (aggregate)†		Median (%) resistant (reported) ‡	Range (for labs reporting resistance)	
	Testing for antimicrobial resistance	Reporting resistance to agent	% Resistant	# Resistant/total # isolates tested		# Isolates tested§	% Resistant (reported)
Ampicillin∝	18	18	98	(1900/1939)	100	NR - 499	67 - 100
Ampicillin/ Sulbactam	15	12	15	(246/1624)	11	NR - 499	3 - 30
Amikacin	14	0	0	(0/1490)	0	--- ---	--- ---
Cefazolin	21	17	5	(105/2270)	2	NR - 499	1 - 33
Cefotetan	10	3	1	(10/1264)	0	59 - 499	1 - 3
Cefoxitin	4	2	2	(16/838)	1	147 - 499	1 - 3
Ceftazidime	18	4	1	(12/1948)	0	59 - 499	1 - 3
Ceftriaxone	19	3	0	(5/1908)	0	6 - 145	1 - 33
Cefuroxime	13	10	6	(90/1516)	5	21 - 499	1 - 20
Ciprofloxacin	20	9	1	(22/2270)	0	21 - 499	1 - 5
Gentamicin	21	6	1	(15/2270)	0	125 - 499	1 - 2
Imipenem	15	2	0	(3/1833)	0	38 - 125	1 - 5
Levofloxacin	16	8	1	(30/2107)	1	21 - 499	1 - 10
Piperacillin∝	14	12	7	(113/1641)	4	24 - 499	2 - 36
Piperacillin/ Tazobactam	10	8	4	(66/1546)	3	NR - 499	2 - 9
Tetracycline	10	8	13	(195/1466)	10	21 - 499	8 - 30
Tobramycin	18	4	0	(6/2127)	0	125 - 209	1 - 1
Trimethoprim/ Sulfamethoxazole	20	16	5	(119/2232)	3	NR - 499	3 - 14

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
§NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.
∝The tests for this antimicrobial agent may not appropriate to report (per NCCLS) or use for clinical decision-making. However, the result was reported on the antibiogram by the reporting laboratory, it may or may not have been reported to the physician by the laboratory.

Pseudomonas aeruginosa *Pseudomonas aeruginosa* has intrinsic resistance to most available antibiotics, leaving aminoglycosides, anti-pseudomonal penicillins, newer cephalosporins, imipenem and flouroquinolones as treatment options for systemic infection. *Pseudomonas* is ubiquitous in the hospital, frequently colonising patients before admission and contaminating water and various foods. The pseudomonads are better known to microbiologists as pathogens of plants rather than animals, but three Pseudomonas species are pathogens of humans. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in patients with severe burns, and in cancer and AIDS patients who are immunosuppressed. *Pseudomonas aeruginosa* is occasionally a pathogen of plants as well.

The levels of resistance to select antimicrobials for *Pseudomonas aeruginosa* are presented in Table 12.

Table 12. Antimicrobial resistance patterns reported by Montana laboratories for *Psuedomonas aeruginosa* isolates, 2002.

Antimicrobial Agent	Number of laboratories		% Resistant (aggregate)†		Median (%) resistant (reported) ‡	Range (for labs reporting resistance)	
	Testing for antimicrobial resistance	Reporting resistance to agent	% Resistant	# Resistant/total # isolates tested		# Isolates tested§	% Resistant (reported)
Ampicillin∝	11	11	99	(1215/1223)	100	10 - 342	97 - 100
Ampicillin/ Sulbactam∝	7	7	97	(995/1031)	99	1 - 216	92 - 100
Amikacin	13	10	6	(91/1551)	2	1 - 342	1 - 100
Ceftazidime	17	14	12	(226/1909)	11	NR - 342	6 - 23
Ciprofloxacin	19	17	27	(550/2008)	23	10 - 342	6 - 37
Gentamicin	20	15	19	(388/2008)	10	10 - 342	6 - 38
Imipenem	14	13	12	(212/1801)	9	23 - 342	6 - 20
Levofloxacin	16	14	27	(428/1599)	23	NR - 237	6 - 37
Meropenem	4	2	4	(10/243)	0	NR - 123	8 - 12
Piperacillin	14	12	7	(132/1762)	5	NR - 342	3 - 100
Piperacillin/ Tazobactam	11	8	4	(54/1493)	1	NR - 342	1 - 17
Tobramycin	17	10	6	(106/1786)	1	64 - 342	1 - 16

†Calculated using only data from laboratories that provided numbers of resistant organisms and number of organisms tested for antimicrobial resistance.
‡Calculated as the median percent resistant for all laboratories that reported resistance to this agent.
§NR denotes a laboratory did not report the number of isolates tested for resistance to this agent.
∝The tests for this antimicrobial agent may not appropriate to report (per NCCLS) or use for clinical decision-making. However, the result was reported on the antibiogram by the reporting laboratory, it may or may not have been reported to the physician by the laboratory.

Conclusions, Limitations, and Recommendations

In 2003, the State of Montana Department of Public Health and Human Services Public Health Laboratory (PHL) surveyed laboratories in the State of Montana regarding antimicrobial susceptibility testing and antimicrobial resistance patterns observed. This survey was undertaken as a follow-up to a similar survey conducted in 1996 that collected data about reported AMR patterns during that year. To maximize the response rate for the survey, a two-tier approach was used. First, the questionnaire was sent to 76 laboratories throughout the state. A follow-up telephone call was placed to further prompt those laboratories that did not respond to the questionnaire or returned the questionnaire without an antibiogram attached.

This process resulted in an overall response rate of 72.4%, making this survey representative of laboratories throughout the state. Of the laboratories that performed microbiology, 59% submitted an antibiogram. Although antibiograms from a higher proportion of responding laboratories would be instrumental in strengthening the representativeness of this data, this information remains valuable in estimating the levels of resistance throughout the state. Further improvements in administering the survey and gathering antibiogram data will be considered in future endeavors based on the lessons learned from the previous years work.

Most laboratories that report performing AST are using automated MIC testing. However, few laboratories are performing confirmatory testing on organisms with potentially significant resistance when it does occur. In addition, unusual reports of resistance (i.e VRSA) continue, although with less frequency than previously reported. Unfortunately, it is impossible from the nature of this survey to determine if the reporting laboratory took action based on these findings or what those actions were (i.e. secondary testing, referral). In any event, a follow-up investigation of these reports would be prudent to establish their validity. In addition, further education of laboratory personnel may be warranted as to the appropriate action steps in the event of reporting unusual results.

While virtually all laboratories that responded to the survey use NCCLS documents to establish their reporting rules, only approximately one-third of the laboratories actually have access to the manuals. However, the majority of laboratories utilize automated AST technologies that most likely have the NCCLS standards built-in; therefore, labs have indirect access to these important criteria. However, those laboratories that do not have access to NCCLS documents are invited to contact the Montana Department of Public Health Laboratory for copies of these documents.

Many laboratories in Montana have small staffing; the number of staff trained to perform AST ranged from 2 – 12 persons; of these staff, a high proportion are likely to be cross-trained to perform laboratory testing in areas other than microbiology. This finding may have ramifications for smaller laboratories whose personnel are attempting to maintain proficiencies in multiple areas of laboratory work.

For *Steptococcus pneumoniae*, significant levels of resistance to penicillin continues to reinforce that all isolates should be tested. However, culture submission or isolate testing practices may play a role in the wide variability among the percent resistance reported. Further refinement of future surveys to address this variability are warranted to ensure clinically relevant conclusions can be ascertained from this data.

High levels of penicillin-resistant *S. aureus* were reported; however, this may reflect a sampling bias for this organism due to inclusion of screening isolates. The wide variability in the percent resistant (reported) would suggest this may be true. Further refinement of future surveys to investigate the effect of including screening isolates in the facility antibiogram (and subsequently the "state" antibiogram) is merited.

Of the laboratories that test enterococci for resistance to vancomycin, one-third reported finding resistance; however, the overall percent resistant remains low. For this survey laboratories reported resistance patterns for all *Enterococci spp*.; therefore, it is unknown if species that are intrinsically resistant to antimicrobial agents influence these findings. To address the effect of species on the antibiogram findings, future surveys may need to more precisely identify the Enterococci species of interest.

Less than one-third of laboratories that tested for ESBLs reported finding them and very low levels of ESBLs were reported.

Levels of antimicrobial resistance appears to be relatively stable for *S. pneumoniae, S. aureus*, and *E. coli* over the 1996 – 2002 six-year time period, with a few aforementioned exceptions. However, due to the non-specific nature of the surveys, these results may reflect differences in testing methodologies, culture submission practices, changes in antimicrobial usage patterns, true changes in resistance levels over time, or a combination thereof. Future surveys must be constructed with comparative, time-trend data analyses in mind so that the aforementioned limitations in interpretation can be adequately addressed. Given the potential changes in resistance that are noted and the potential serious public health consequences of resistant infections, these trends in antimicrobial resistance merit further and continued monitoring on a state-wide basis.

Appendix I

Montana Department of Public Health and Human Services
Public Health Laboratory

Antimicrobial Susceptibility Testing (AST) Survey

Return survey by April 14, 2003: fax to 406-444-1802, or mail to
Ginny George, Public Health Laboratory, P.O. Box 6489, Helena, MT 59604

Responding Laboratory: ________________________________

Responsible Individual: ________________________________

Telephone: _______________ e-mail: ______________________

Questions	*S. pneumoniae*	***Enterococcus sp.***		***S. aureus***		***E. coli / K. pneumoniae***		***P. aeruginosa***
Number of isolates from 1/1/02 to 12/31/02 for which susceptibility testing was performed	**Blood & CSF**	**Blood/CSF**	**Other**	**Blood/CSF**	**Other**	**Blood/CSF**	**Other**	**Blood & CSF**
What primary method(s) does your lab use to test for antibiotic susceptibility? (Mark all that apply)	○ MIC, automated ○ MIC, manual ○ Oxacillin Disk for Penicillin Resistance ○ Etest	○ MIC, automated ○ MIC, manual ○ Disk Diffusion ○ Etest		○ MIC, automated ○ MIC, manual ○ Disk Diffusion ○ Etest		○ MIC, automated ○ MIC, manual ○ Disk Diffusion ○ Etest		○ MIC, automated ○ MIC, manual ○ Disk Diffusion ○ Etest
What secondary method(s) are used to test for or confirm antibiotic susceptibility? (Mark all that apply)	○ Disk Diffusion ○ Other (specify) _______________	○ Vancomycin Screening Agar ○ Vancomycin Disk Diffusion ○ Vancomycin Etest ○ Other (specify) _______________		○ Oxacillin Salt Agar Screen ○ Vancomycin Screening Agar ○ Disk Diffusion ○ Etest ○ Other (specify) _______________		○ Disk Diffusion for ESBLs w/ wo Clavulinic Acid ○ Etest for CT/CTL, TZ/TZL ○ Other (specify) _______________		○ Other (specify) _______________

►**Please enclose a copy of your antibiogram from 2002, and your reporting rules**, if available. Antibiogram attached ○ Yes ○ No
Reporting Rules attached ○ Yes ○ No

►How are reporting rules made/decided upon? (mark all that apply) ○ NCCLS documents ○ Pharmacist Cooperation ○ ID Physician Cooperation
○ Reporting only drugs on Institution's Formulary ○ Other (specify) ______________________________

►Does your laboratory have the current (January 2003) NCCLS documents listed below?

M2-A8 - Disk diffusion ○ Yes ○ No ○ On Order **M7-A6 – MIC** ○ Yes ○ No ○ On Order
M100-S13 ○ Yes ○ No ○ On Order **M39-A** ○ Yes ○ No ○ On Order

►What was the date of the last training session or self-study course you or your staff attended regarding Antimicrobial Susceptibility Testing (AST)?
Date: ____________________ Session: ______________________________

►List the total number of staff (not FTE) trained to do AST at your facility: __________

►What percentage of these staff perform laboratory testing in other laboratory areas in addition to Microbiology (cross-trained): ________